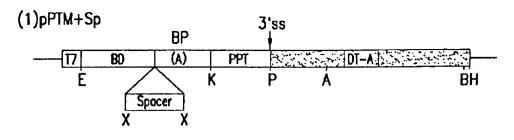
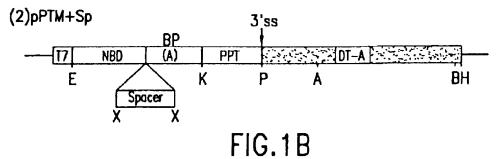


FIG. 1A





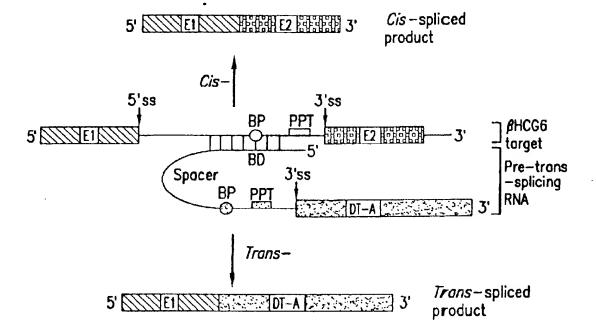


FIG.1C

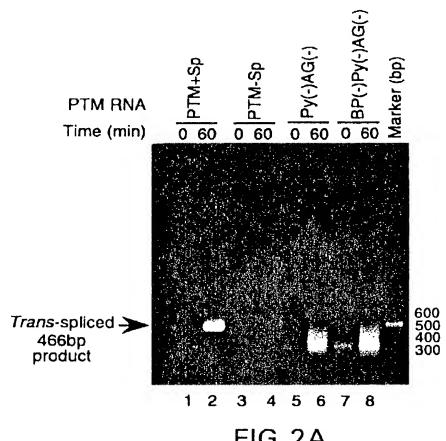
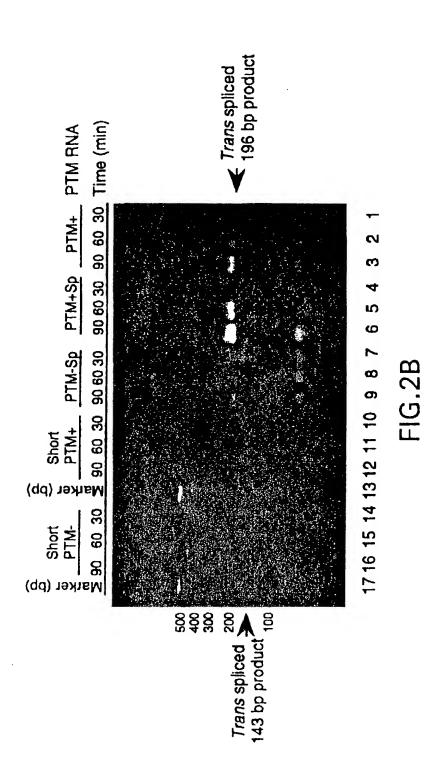


FIG.2A



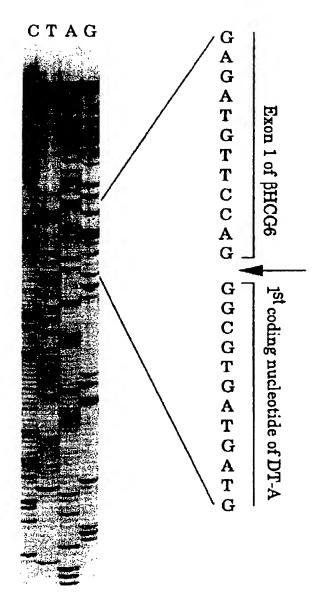


FIG.3

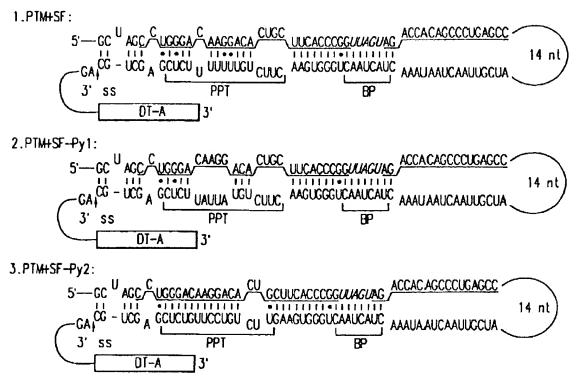


FIG.4A

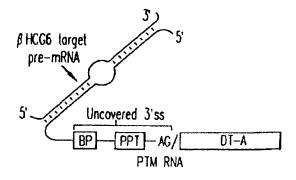


FIG.4B

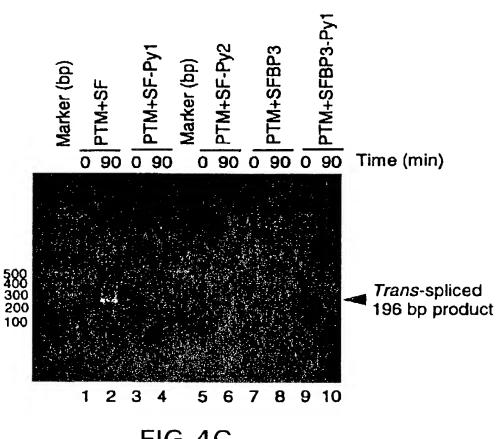
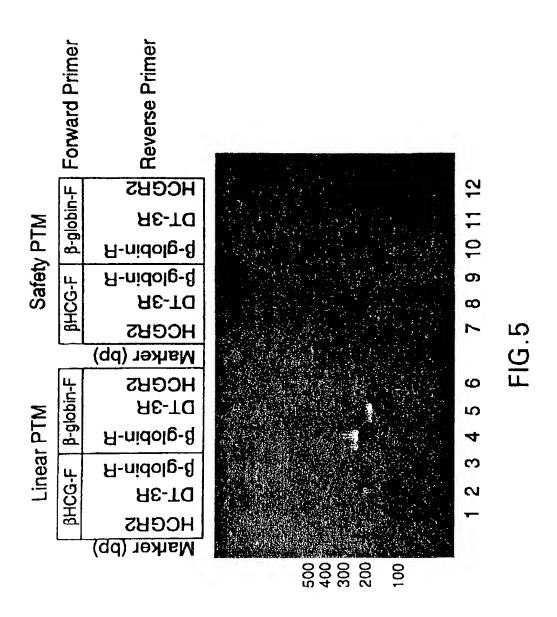
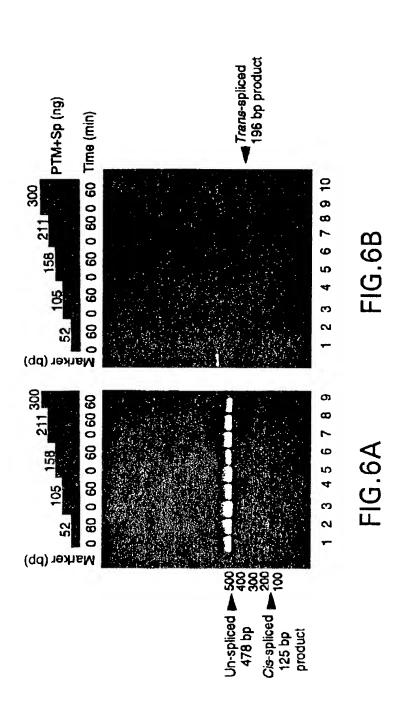
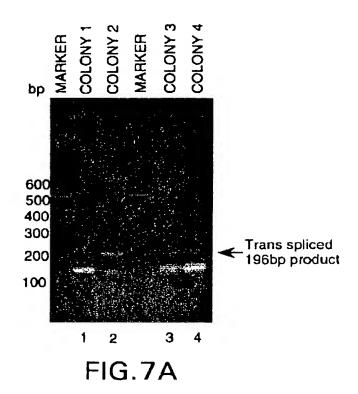


FIG.4C



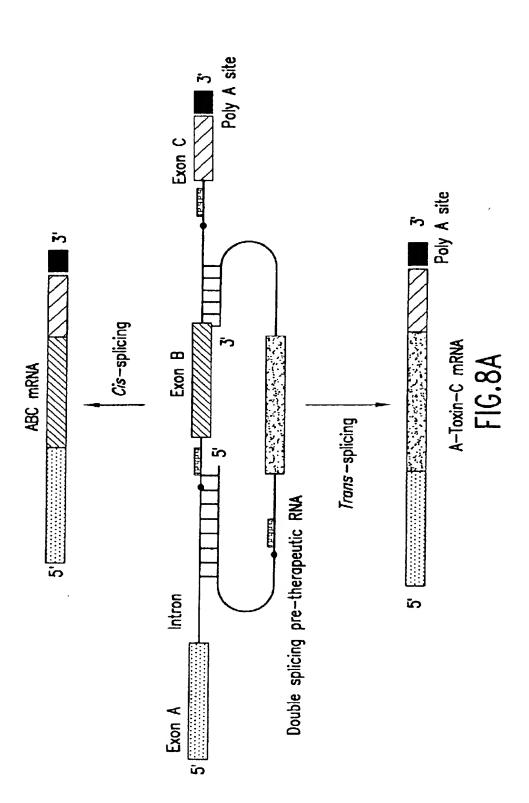


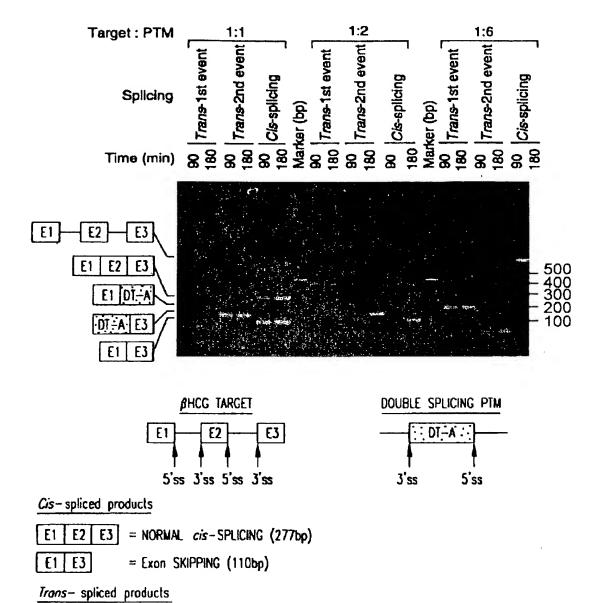


EXON 1 OF PHOSE | 5-CAGGGGACGCACCAAGGATGTTCCAG-GGGGCTGATGTTGTT
| 1ST CODING NUCLEOTIDE OF DT-A
GATTCTTCTTAAATCTTTGTGATGGAAAACTTTTCTTCGTACCAGGGACTA

AACCTGGTTATGTAGATTCCATTCAAAA-3'

FIG.7B





007945249356.0109844

FIG.8B

E1 DT-A = 1st EVENT, 196bp. Trans-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. Trans- SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

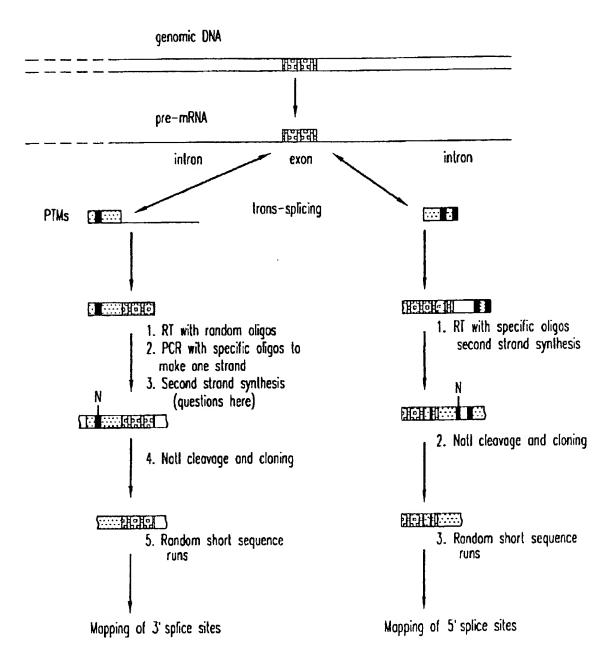
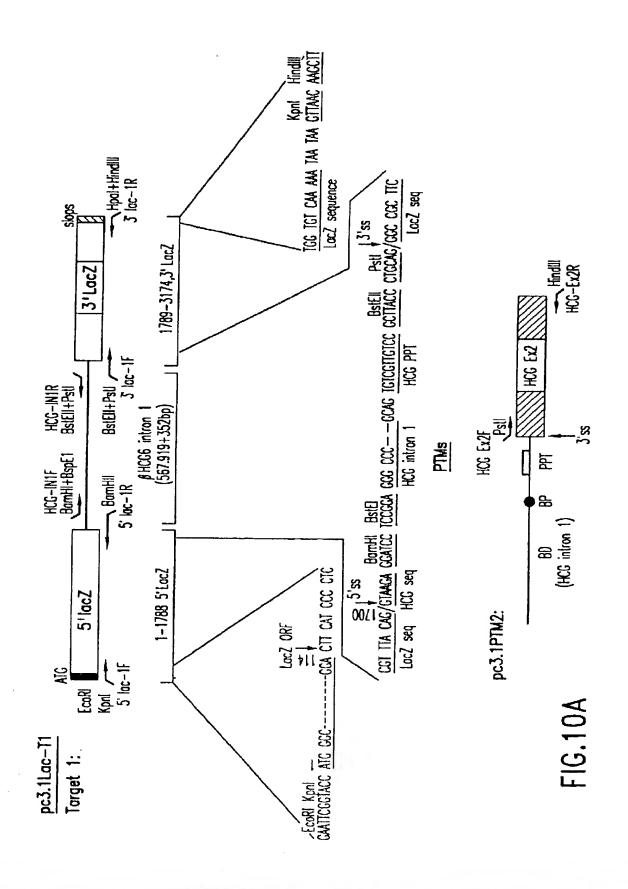


FIG.9



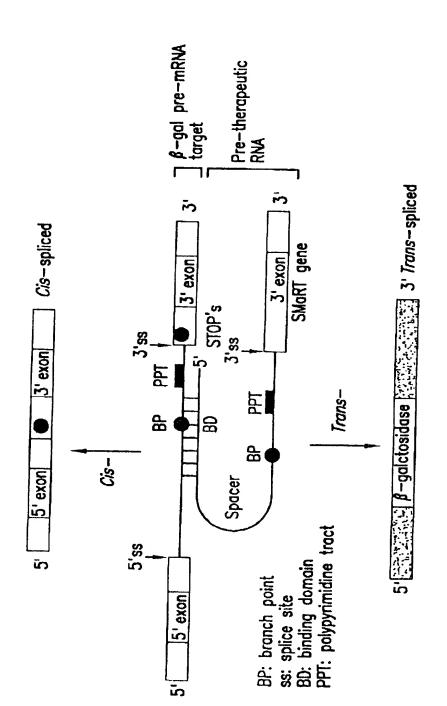
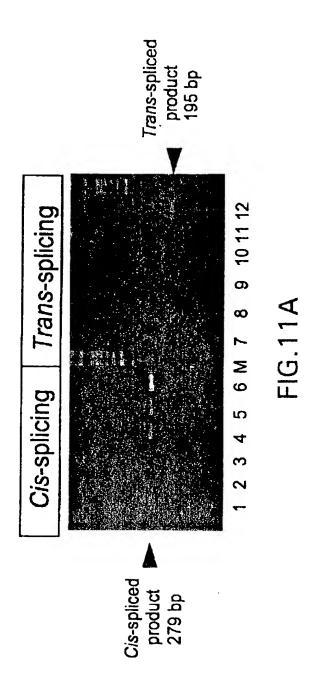


FIG. 10B



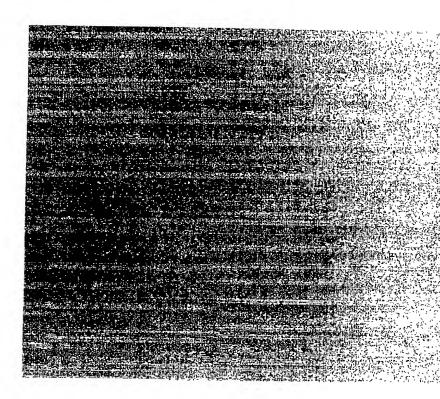


FIG.11B

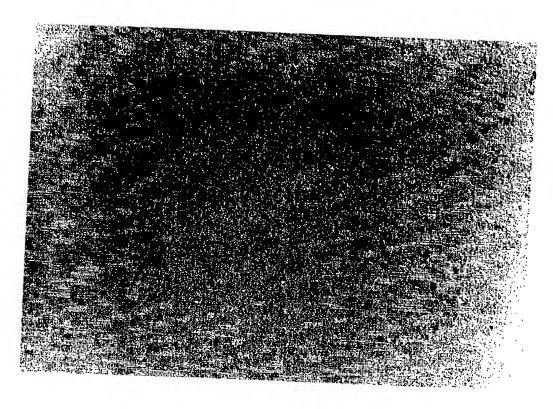


FIG.11C

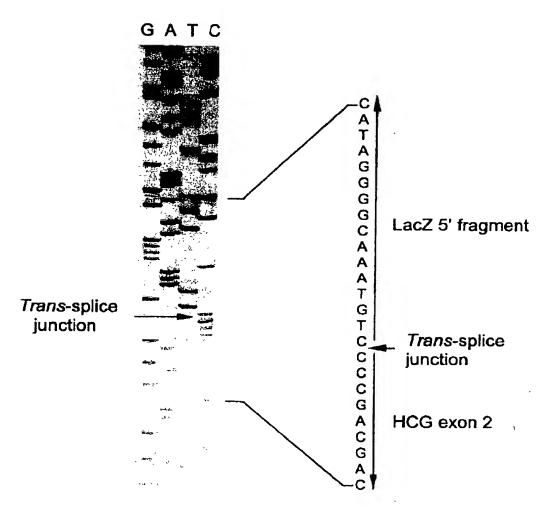


FIG.12A

1. NUCLEOTIDE SEQUENCES OF THE cis-SPLICED PRODUCT (285 bp):

BioLac-TR1

GCCTTTCCCTCCTCCAGAGACCCCCCCTGATCCTTTGCGAATACCCCCACCCGATGGGTAACAGTCTTG

GOGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCCGTTTACAG/GGCGCCTTCGTCTAATAATG Splice junction

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTT

TOSCOSATACCCCCAACCATCCCCAGTTCTGTATGAACCGTCTGGTCTTTGCCCACCCCACCCCATCCAG

2. NUCLEOTIDE SEQUENCES OF THE trans-SPLICED PRODUCT (195 bp)

BioLac-TR1

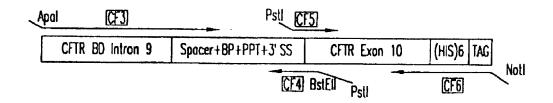
GCCTTTCGCTACCTGGAGAGACGCCCCCTGATCCTTTGCGAATACGCCCACGCGATGCGTAACAGTCTTGG

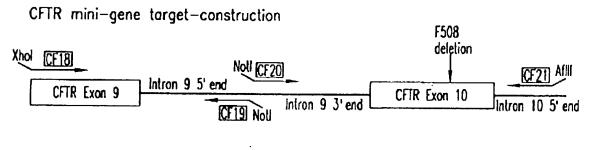
CGGTTTCCCTAAATACTCCCAGGCGTTTCGTCAGTATCCCCGTTTACAG/CGCCTGCTGCTGTTCCTGCTGCTGCT Splice junction

GAGCATGGGGGACATGGGCATCCAAGGAGCCACTTCGGCCACGAGGTGCCG

FIG. 12B

CFTR Pre-therapeutic molecule (PTM or "bullet")





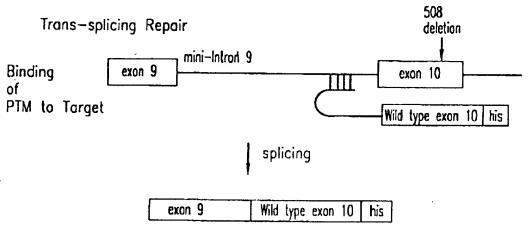


FIG.13

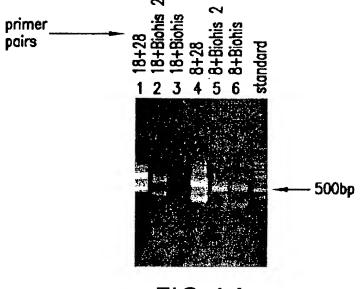


FIG.14

Kpn I Pst I 3'ss I CGTACCTCTCTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGCTAAAAT 160 TAAGCACAGTGGAAGAATTTCATTCTGTTCTCAGTTTTCCTGCATTATGCCTGGCACCATTAAAGAAAATATCATCTTTG 240 GIGITICCTATGATGATAGATACAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGCATCATCATCATCATCATTAG |320 GCTAGCGITTAAACGGGCCGACCCATCATTATTAGGTCATTATCCGCGGAACATTATAAACGTTGCTCGAGTACTAAC 80 CGATCCCAAATTTGCCCGGGGGGTGGTAGTAATAATAATAGGCGCCTTGTAATATATTGCAACGAGCTCATGATTG ACCATGGAGAAGAAAAAAAGGACGTCHGAAGTGAAGATTACTACTAATACCCTCTTGACCTCGGAAGTCTCCCATTTTA ATTCGTGTCACCTTCTTAAAGTAAGACAAGACTCAAAAGGACCTAATACGGACCGTGGTAATTTCTTTTATAGTAGAAAC CACAAAGGATACTACTIATATCTATGTCTTGGCAGTAGTTTGGTACGGTTGATCTTCTCGTAGTAGTAGTAGTAATC Positions of Restriction Endonucleases sites (unique sites underlined) DNA sequence 500 b.p. GCTAGCGTTTAA ... TGCCACTCCCAC linear BINDING DOMAIN INTRON 9 BD 102 Hoe III Sau96 I Sau96 1 Ban 11 Apa Nhe I Dra I

FIG. 15A

				.158
Sau 3 Sau	378 PRESENT IN PTM 3' UT Sau3A 1 378 BUT NOT TARGET ppn 1	TAAACCCTGATCAGCCTCCACTTCTAGTTGCCAGCCATCTGTTTGCCCCTCCCCCTTCCTT	CTGCAAGCTGCCACTCCCAC 500 GACCTTCCACGGTGAGGGTG	Restriction Endonucleases site usage
Hae ill Not 1 	v, Δ-	TAAACCGCTG ATTTCCCCAC	CTCCAACCTCC GACCTTCCACC	

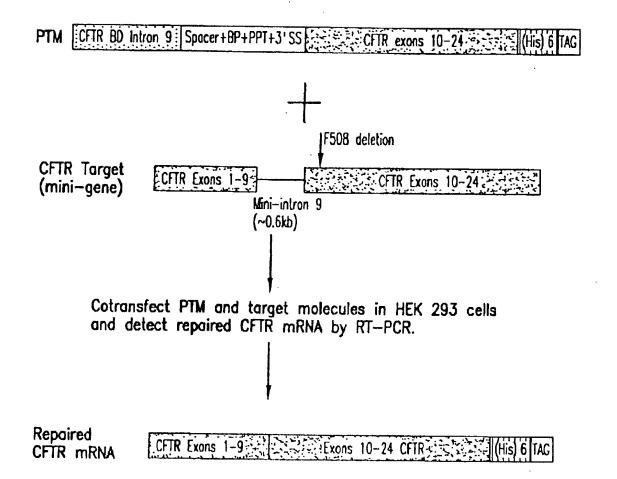


FIG. 16

Double Splicing PTM

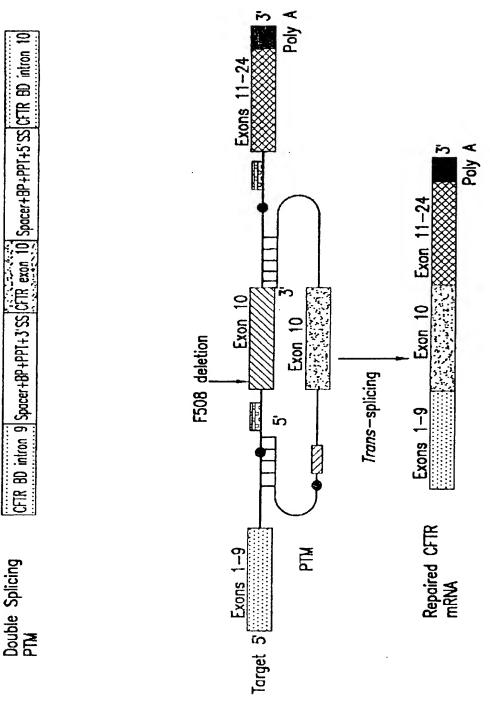
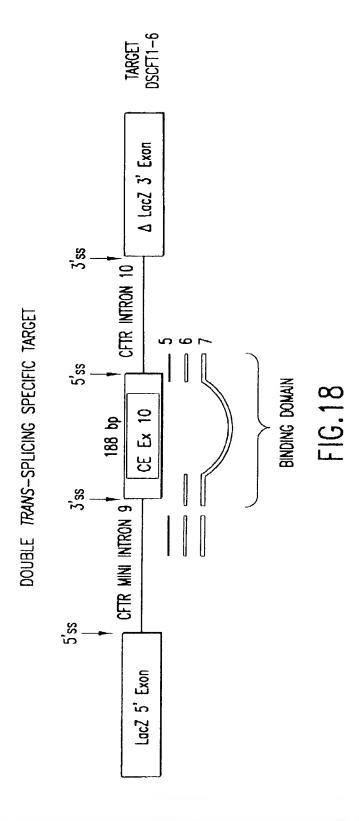


FIG.17



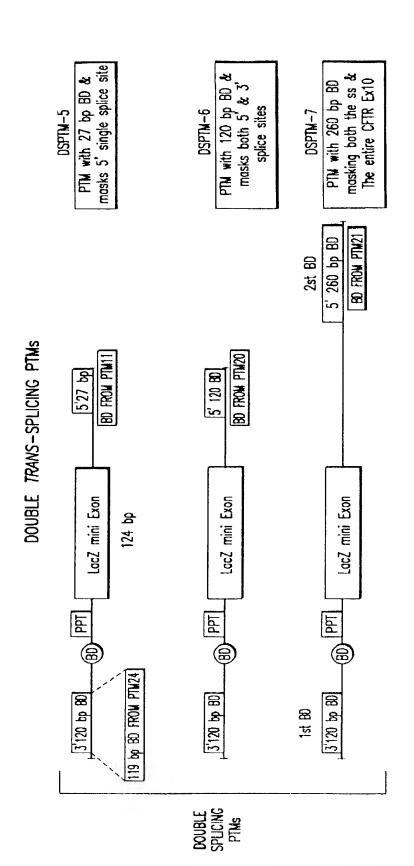
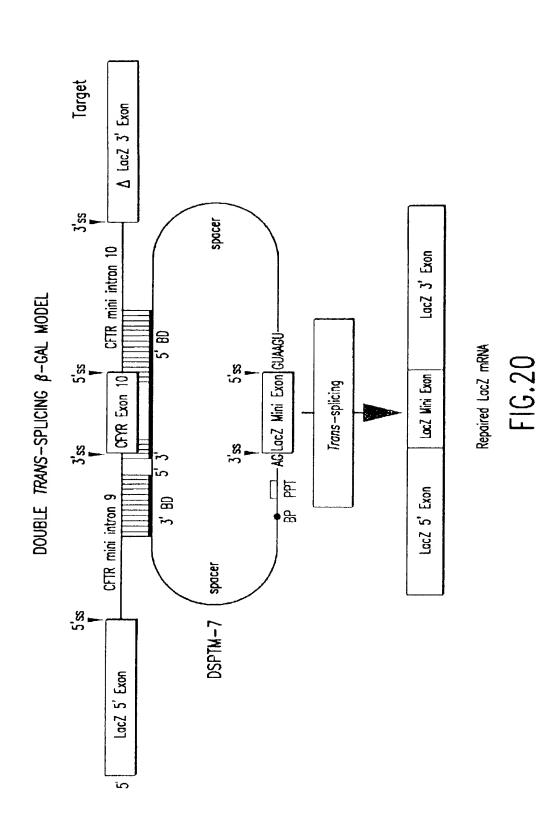
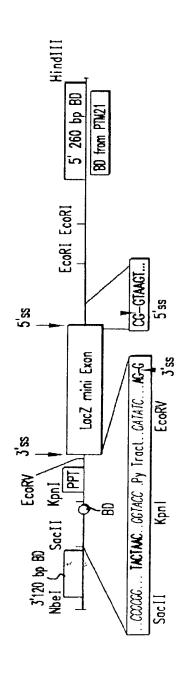


FIG. 19





(1) 3' BD (120 BP): CATICACTICCICCAATTAICATCCTAAGCACACTGIATATTCTIATTIGTAAAGATICTATTAACTCATTTGATTC AAAATATTTAAAATACTTCCTGTTTCATACTCTGCTATGCAC

(2) Spacer sequences (24 bp): AACAITAITATAACCITGCICCAA

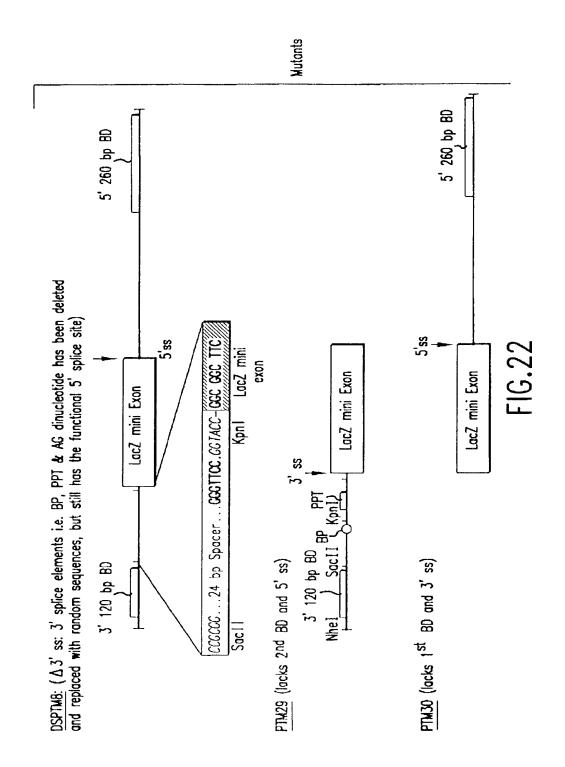
(3) Branch point, pyrimidine tract and acceptor splice site: IACIAAC I GCIACC ICTICTITITITITICAIAIC CIGCAC GCC LacZ mini 5'ss

3'ss LacZ mini

(4) 5' donor site and 2nd spacer sequence: | IGA ACG|67AAG1 GTTATCACCCATATGTGTGTCTAACCTGATTGGGGCCTTCGATACG CTAAGATCCACCGG BD (260 BP): ICAAAAAGITITCACATAATTICTTACCICTICTICAAATGATGCTTTGATGACGCTTCTGTATGTATTCATCATTGCAA 2 (5)

ACACCAATGATTTTTETTTAATGGTGCTGGCATAATCCTGGAAAACTGATAACAATGAAATTETTCCACTGTGGTTAA AAAAACCCTCTGAATTGTCCCATTTGTCCCATAATCATCATTACAACTGAACTCTGGAATAAAACCCATCATTATTAACTCA TTATCAAATCACGC

FIG.21



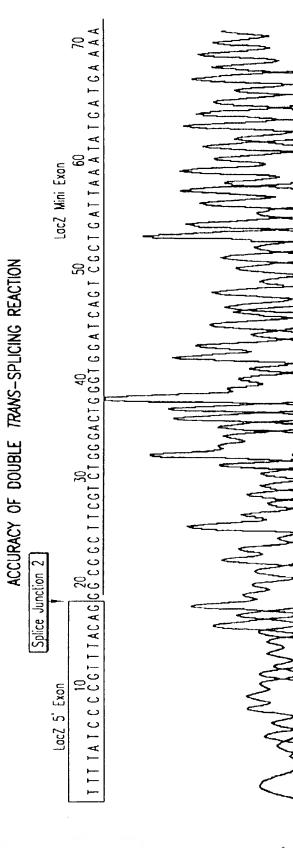


FIG.23A

LacZ 3' Exon ACCURACY OF DOUBLE TRANS-SPLICING REACTION Splice Junction 2

FIG.23B

Double Trans-splicing Produces Full-length Protein

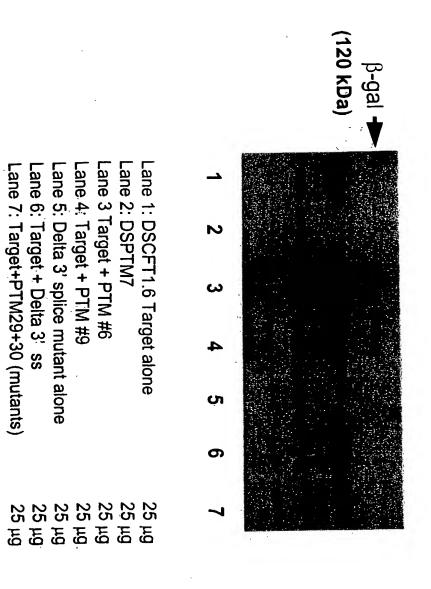
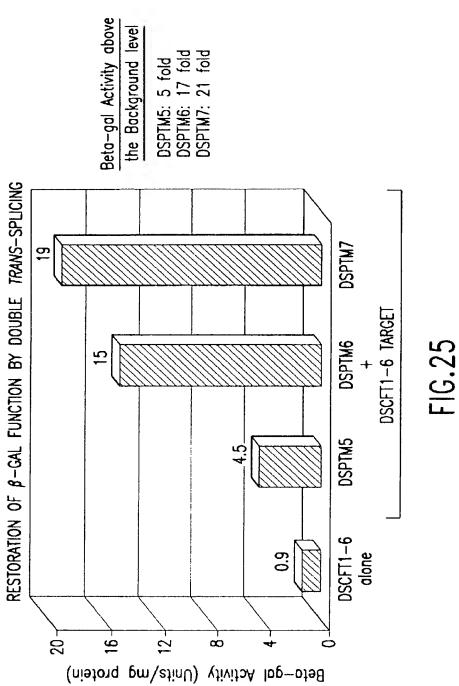


Figure 24



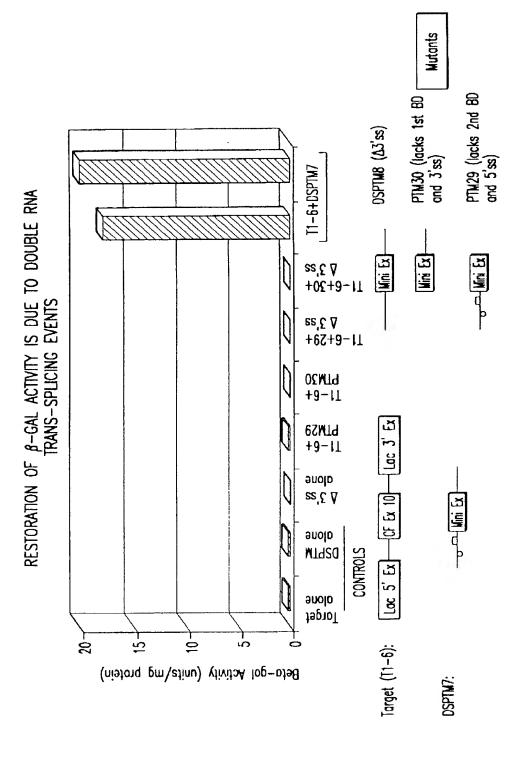
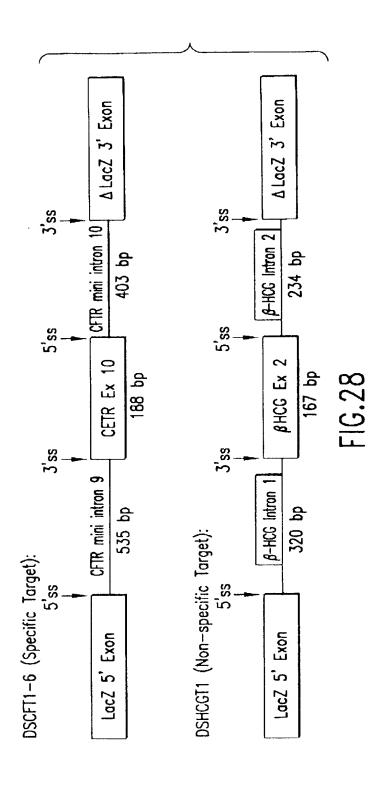


FIG.26

is $\sim 1-1.5\%$ of the best to double trans-splicing beta-gal activity due (3' exon replacement) The current level of single splice model Increasing conc. of DSPTM plus more Target DSPTM7 | 2+1.5 2+3.0 2+4.5 | (3+1.5 3+3.0 3+4.5) DOUBLE TRANS-SPLICING: TITRATION OF TARGET & PTM Increasing conc. of DSPTM aíone CONTROLS Target alone 10-25-20-15 30-33 A-Gal Activity (units/mg protein)

FIG.27



SPECIFICITY OF DOUBLE TRANS-SPLICING REACTION

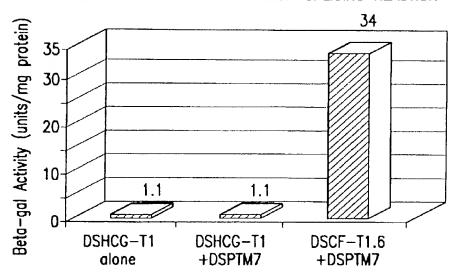


FIG.29

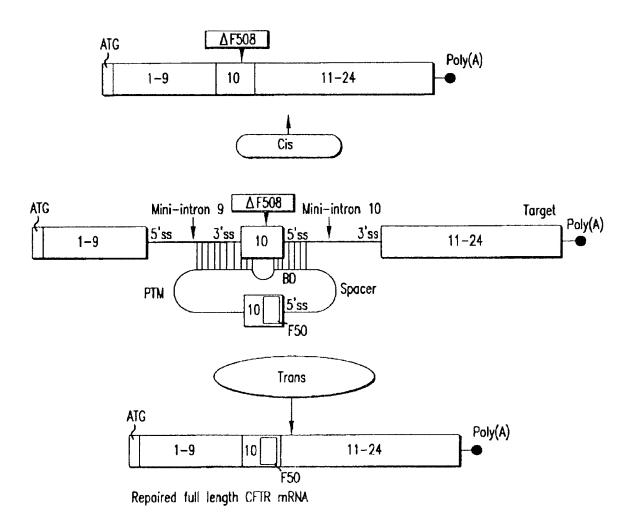


FIG.30

PTM with a long binding domain masking two splice sites and part of exon 10 in a mini-gene target

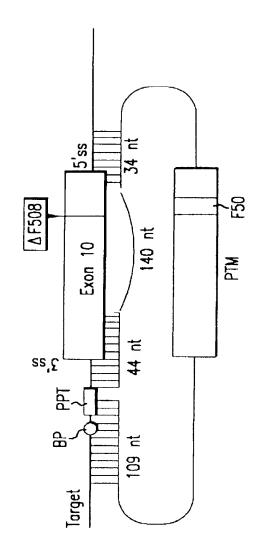


FIG. 31

Sequence of a double *Trans*—spliced product

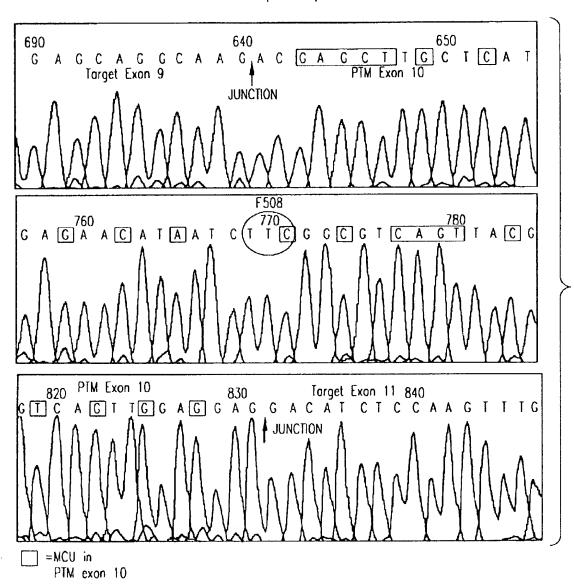
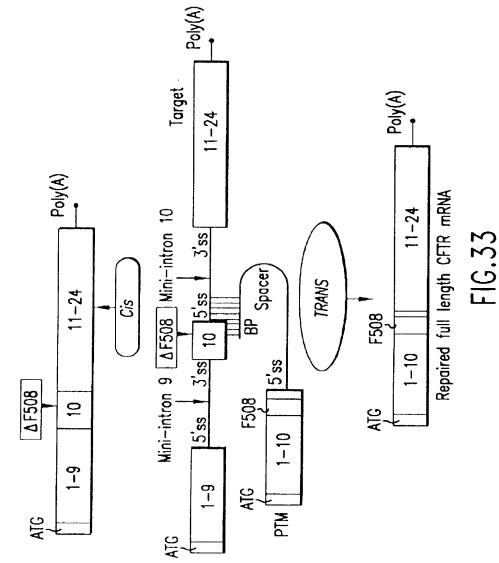


FIG.32

CF—TR Repair: 5' Exon—Replacement schematic diagram of a PTM binding to the splices site of intron 10 of a mini—gene target



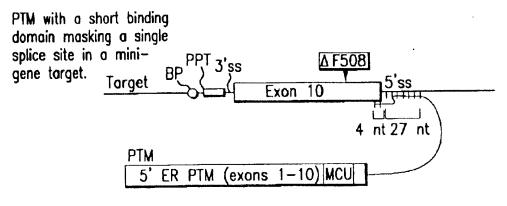


FIG.34A

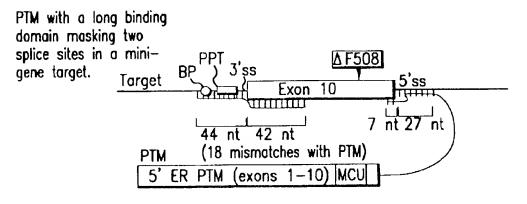


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini—gene target.

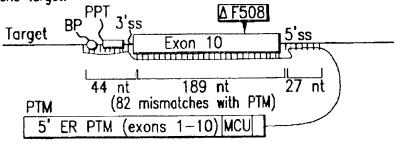
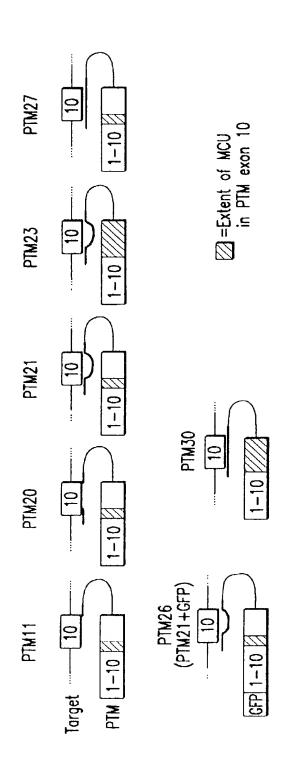


FIG.34C

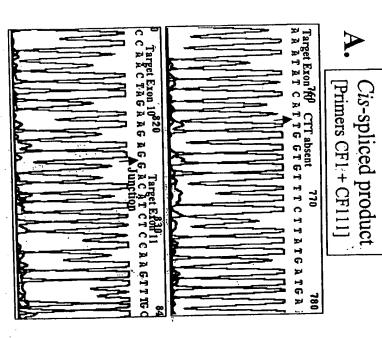


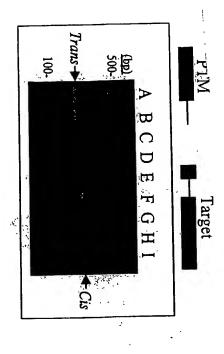
MCU in exon 10 of PTM 88 of 192 (46%) bases in PTM exon 10 are not complemetary to its binding domain.

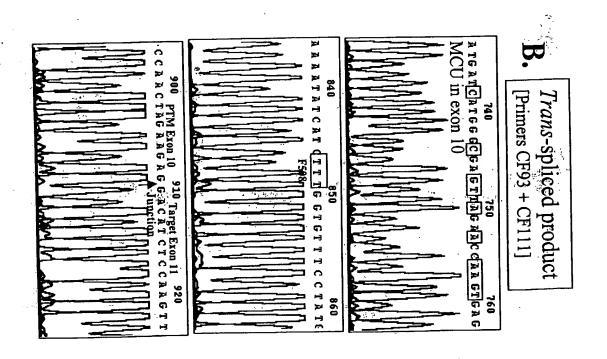
ACCACCTTCCTCATCATCATCATCCCCCACTTACAACCCAACTCAACCCAAGATCAAACATTCCC GCCCCATCACCTTTTCCACCCAATTCACTTCCATCATCACCTACCATCAACATAAT CTTCCCCTCACTTACCACCAGTACCCCTATCCCTCCTCATTAAGCCCTCTCACTTCCAGCAGACACATAAT

FIG.35

Figure 36







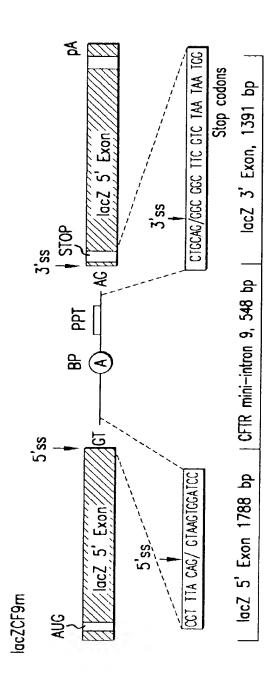


FIG.37A

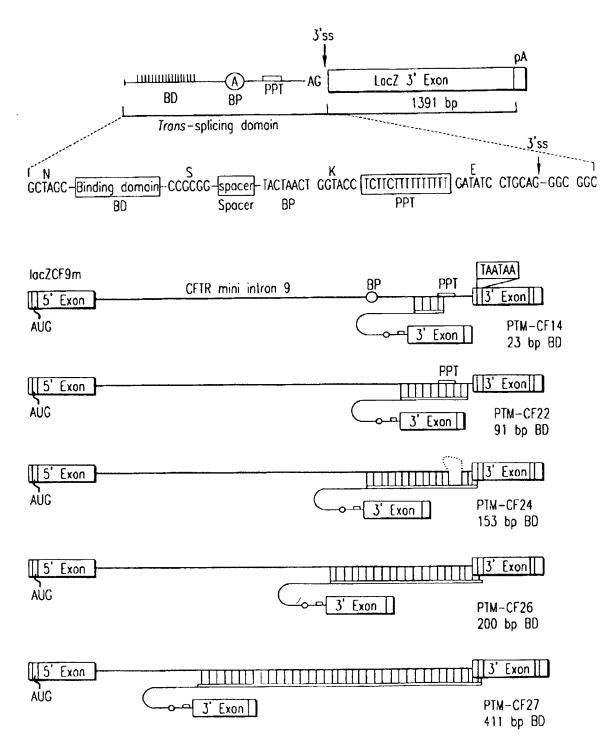
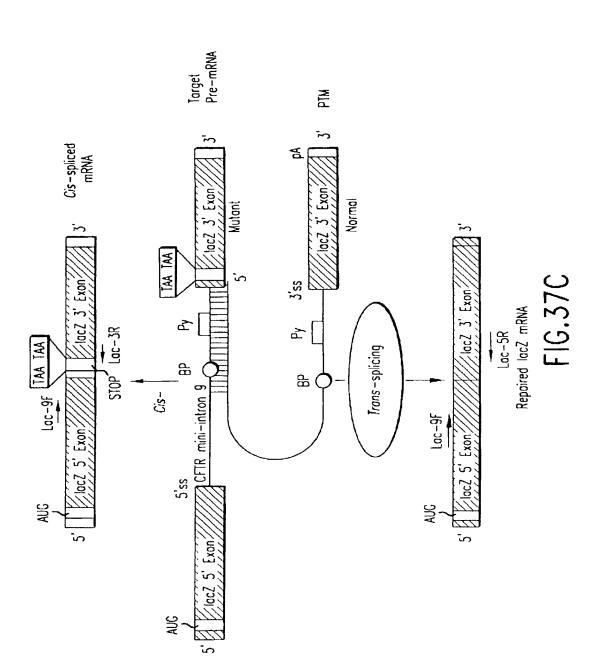
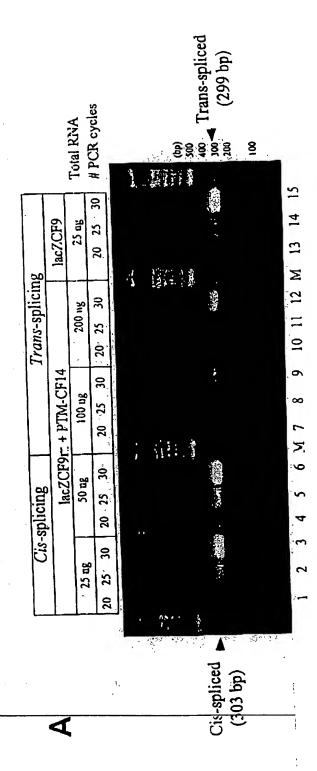


FIG.37B





top)
soc 400
soc Trans-spliced
200 (299 bp) # PCR cycles Total RNA 8 14 15 30 50 ng lacZCF9 20 25 13 10 11 12 M Trans-splicing ္က 100 ng ฆ 20 6 lac2CF9n1 + PTM-CF24 39 00 50 ng 20 25 ~ Σ v 25 30 Sı) IIB Cis-splicing 2 2 25 ag 25 20 1000年 ¢is-spliced (303 bp)

Figure 35 A

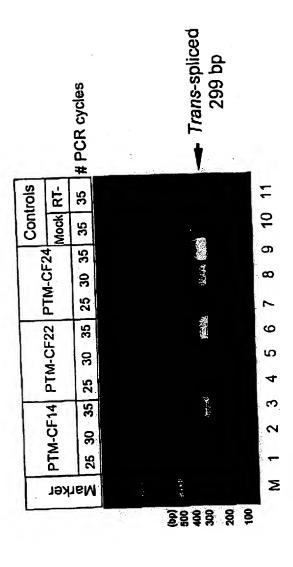


Figure 38B

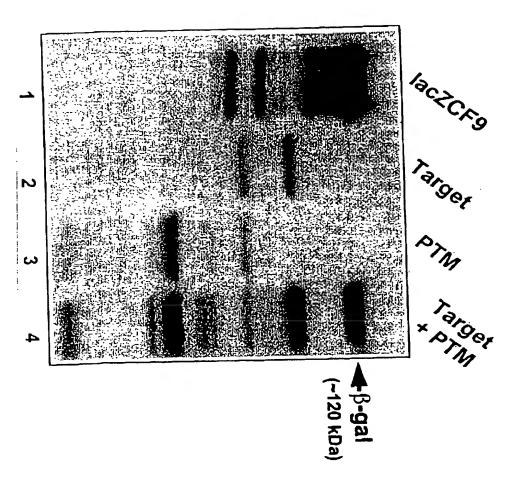


Figure 39

00000100 0000 01

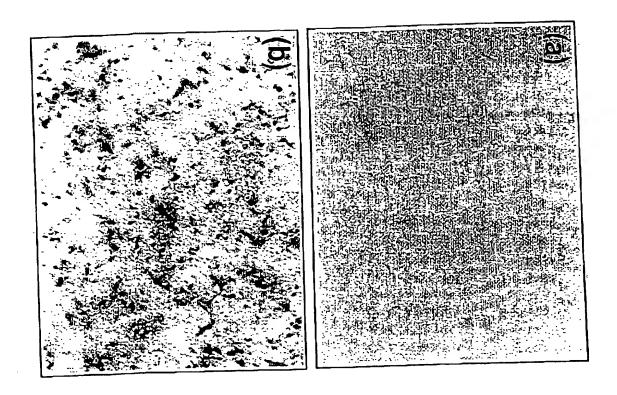
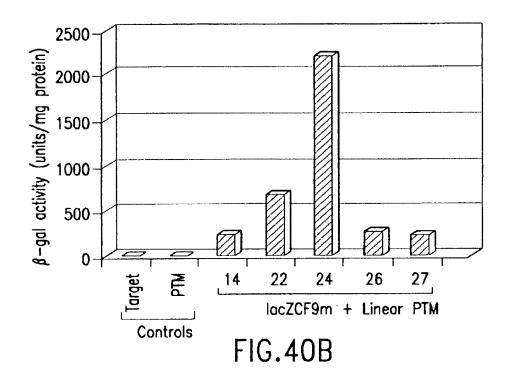
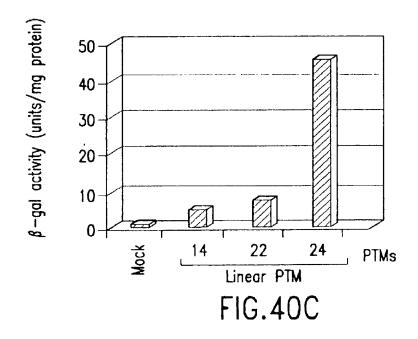


Figure 40A





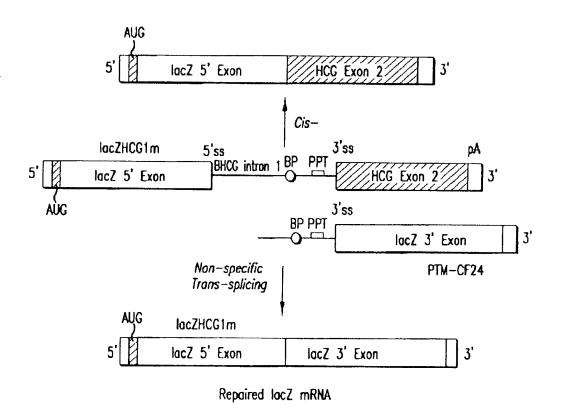
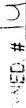


FIG.41A



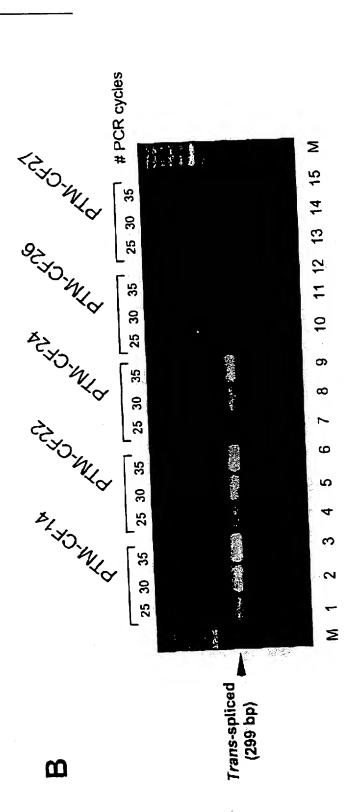


Figure 410

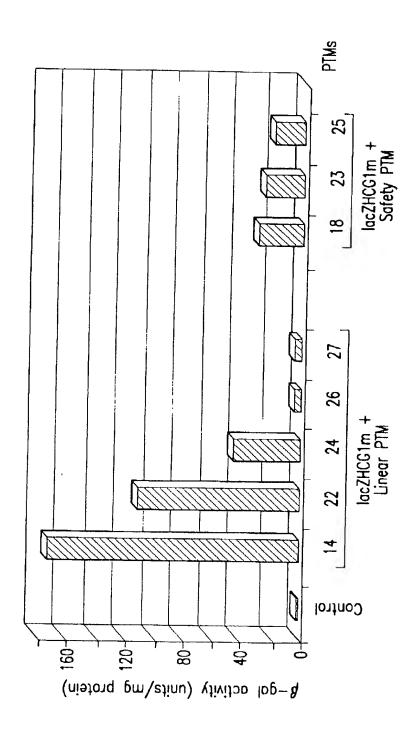


FIG.41C

Exons

1-10

ATGCAGAGGTCGCCTCTGGAAAACGCCAGCGTTGTCTCCAAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG GGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTAATGCCCTTCGGCGATGTTTTTTCTGG AGATTTATGTTCTATGGAATCTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA TAGCTTCCTATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTTATCTAGGCATAGGCTTATGCCTTCTCTTTAT TGTGAGGACACTGCTCCTACACCCAGCCATTTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAGCTATGTTTAGT TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACTTGTTAGTCTCCTTT CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCACATTTCGTGTGGATCGCTCCTTTGCAAGTGGCACTCCT CATGGGGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCCTGATAGTCCTTGCCCTTTTTCAG GCTGGGCTAGGGAGATGATGATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGATTACCTCAG AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGAAAAAATGATTGAAAACTTAAGACA AACAGAACTGAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTTCTTT GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGGAAAATATTCACCACCATCTCATTCT GCATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAGCAATAAA CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGCAG AATGTAACAGCCTTCTGGGAGGAGGATTTGGGGAATTATTTGAGAAAAGCAAAACAATAACAATAGAAAAACTT CTAATGGTGATGACAGCCTCTTCTTCAGTAATTTCTCACTTCTTGGTACTCCTGTCCTGAAAGATATTAATTTCAAGAT TTAGAACCAAGTGAAGGCAAGATCAAACATTCCGGCCGCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTA CCATCAAGGAGAACATAATC77CGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCTGTCAGTTGGA **G**CAG

Trans-splicing domain

GTAAGATATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACGCTAAGATCCACCGC

ICAAAAAGTTTTCACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACCCTTCTGTATCTATATTCATCATTG GAAACACCAATGATATTTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGT GCTTAATTTTACCCTCTGAATTCTCCATTTCTCCATAATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATT ATTAACTCATTATCAAATCACGCT

FIG.42

153 bp PTM24 Binding Domain:

Nhe I

CTAGC—AATAATGAGGAGGGGGCCCTCAGGCTTCACTTGCCTCCAATTATCATCCTAAGCAGAAGTGTATA

TTCTTATTTGTAAAGATTCTATTAACTCATTTGATTCAAAATATTTAAAATACTTCCTGTTTCACCTACTCTGCTATGC

Sac II

FIG.43A

49793514936 OLDER£7

Exons 10-24

ACTICACTICTAATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGGAAGAATTICATTCT GTTCTCAGTTTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTTCCTATGATGAATATAGATA CAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGAGACATCTCCAAGTTTGCAGAGAAAGACAATATAGTTCTTGGAGAA CGTGGAATCACACTGACTGGACGTCAACGAGCAAGAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTTGTATT TATTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAGAAAAAGAAATATTTGAAAGCTGTGTCTGTAAACTGATGGC AGCAGCTATTTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAAACTCATGGGATGTGATT CTTTCGACCAATTTAGTGCAGAAAGAAGAAATTCAATCCTAACTGAGACCTTACACCGTTTCTCATTAGAAGGAGATGC TCCTGTCTCCTGGACAGAAACAAAAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAAAAGGAAGAATTCTATT CTCAATCCAATCAACTCTATACGAAAATTTTCCATTGTGCAMAGACTCCCTTACAAATGAATGGCATCGAAGAGGGATT CTGATGAGCCTTTAGAGAGAAGCCTGTCCTTAGTACCAGATTCTGAGCAGGGGAGAGGCGATACTGCCTCGCATCAGCGT GATCAGCACTGGCCCCACGCTTCAGGCACGAAGGAGGCAGTCTGTCCTGAACCTGATGACAACGTCAGTTAACCAAGGT CAGAACATTCACCGAAAGACAACAGCATCCACACGAAAAGTGTCACTGGCCCCTCAGGCAAACTTGACTGAACTGGATA TATATTCAAGAAGGTTATCTCAAGAAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT TTTTGATGATATGGAGAGCATACCAGCAGTGACTACATGGAACACATACCTTCGATATATTACTGTCCACAAGAGCTTA ATTITIGICCTAATTIGGTCCTTAGTAATTITICTGGCACAGGTGGCTGCTTCTTTGGTGTGTGTGGCTCCTTGGAA ACACTCCTCTTCAAGACAAAGGGAATAGTACTCATAGTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTC CATACTCTAATCACAGTGTCGAAAATTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCCTCA ACACGTTGAAAGCAGGTGGCATTCTTAATAGATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGCCTCTTACCAT ATTIGACTICATCCAGTIGITATTAATTGTGATTGGAGCTATAGCAGTTGTCGCAGTTTTACAACCCTACATCTTTGTT GCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCCTCCAAACCTCACAGCAACTCAAACAACTGG AATCTGAAGGCAGGAGTCCAATTTTCACTCATCTTGTTACAAGCTTAAAAGGACTATGGACACTTCGTGCCTTCGGACG GCAGCCTTACTTTGAAACTCTGTTCCACAAAGCTCTGAATTTACATACTGCCAACTGGTTCTTGTACCTGTCAACACTG CGCTGGTTCCAAATGAGAATACAAATGATTTTTGTCATCTTCTTCATTGCTGTTACCTTCATTTCCATTTTAACAACAG CAGAACGAGAAGGAAGAGTTGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTGCAGTGGGCTGTAAACTC CAGCATAGATGTGGATAGCTTGATGCGATCTGTGAGCCGAGTCTTTAAGTTCATTGACATGCCAACAGAAGGTAAACCT ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGAAATGCCATATTAGA GAACATTTCCTTCTCAATAAGTCCTGGCCAGAGGGTGGGCCTCTTGGGAAGAACTGGATCAGGGAAGAGTACTTTGTTA TGAACAGTGGAGTGATCAAGAAATATGGAAAGTTGCAGATGAGGTTGGGCTCAGATCTGTGATAGAACAGTTTCCTGGG AAGCTTGACTTTGTCCTTGTGGATGGGGGCTGTGTCCTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG TTCTCAGTAAGGCGAAGATCTTGCTGCTTGATGAACCCAGTGCTCATTTGGATCCAGTAACATACCAAATAATTAGAAG AACTCTAAAACAAGCATTTGCTGATTGCACAGTAATTCTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA AAGCCATCAGCCCCTCCGACAGGGTGAAGCTCTTTCCCCACCGGAACTCAAGCCAAGTGCAAGTCTAAGCCCCAGATTGC

Histidine tog Stop
TGCTCTGAAAGAGGAGACAGAAGAAGAGGGTGCAAGATACAAGGCTTCATCATCATCATCATCATCATTAG